NORMAL RESPIRATION AND INTRAMOLECULAR RESPIRATION.

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"RESPIRATION is essentially the intake of oxygen and the output of carbon-dioxide by living cells. In the higher animals two phases of respiration are distinguished — the *external*, the exchange of gases between the air or water and the blood; and the *internal*, the exchange between the blood, lymph, and the tissues." [1] In plants there is, for the most part at least, only the one phase, the exchange of gases between the air or water and the cells composing the tissues, an exchange which is direct and "external," since it takes place in most cases between the air, whether in the intercellular spaces within the plant, or unconfined and outside the plant body, and the individual cells. Even in the densest tissues, within which the intercellular spaces are small, it is likely that the cells take in free oxygen and give out carbon-dioxide, if not directly from intercellular spaces, then from their neighbors bordering on intercellular spaces. In any case, and in every stage of the process of respiration except the purely mechanical ones, of which only the higher animals are capable, the exchange of gases between the cells and the air takes place in solutions, the oxygen entering and diffusing through, the carbon-dioxide passing out from, the cells only when these gases are dissolved in water.

The object of respiration in plants is not the maintenance of a certain body temperature, together with the production of energy needed for doing work, as in warm-blooded animals. It is merely the production of energy for doing work, as in cold-blooded animals. The average body temperature of plants is, in general, nearly the mean daily temperature of their environment. It will vary within certain limits, the variation being

[1] Pembry, M. S. Chemistry of Respiration, Schäfer's *Text-Book of Physiology*, vol. i (1898), p. 692.

large or small according to the environment. Submersed aquatics will vary least, floating aquatics more, and terrestrial plants most in body temperature, other things being equal. But as the temperature of small, still bodies of water (pools, etc.) may vary considerably, so the body temperature of the organisms living therein will vary, being warmed by the sun and cooling during the night. The body temperature of the larger terrestrial plants is likely to be higher at night (except on the exposed surfaces), and lower in the day, than that of the surrounding air. Owing to the great extent of their surface as compared with their mass, radiation from the larger plants is rapid, and a body temperature independent of their environment could be maintained only at great expense of material laboriously collected and elaborated. Plants work economically, are compelled to do so, and this extravagance is avoided.

Heat is the form in which the energy set free by respiration usually makes itself evident, but it does not necessarily follow that only so much energy is liberated as is recognizable as heat, or that this is the only form in which energy is liberated. Only that energy becomes evident as such which is not at once used. In order to determine the amount of energy liberated in respiration, it is necessary to know and to measure the material products of respiration.

The substances ordinarily taking part and produced in the process of physiological oxidation are the highly complex nitrogenous and non-nitrogenous compounds elaborated by the organism and carbon-dioxide, water, and various small amounts of several other substances. Of these last, oxalic acid is the commonest and most important. Since the production of energy and not of any particular compounds is what is striven for in respiration, and since the substances acted upon by free oxygen are different in different plants and even in different cells of the same plants, the products differ accordingly.

Although the oxidation of nitrogenous matter also takes place, it is mainly the non-nitrogenous contents of the living cell which are involved in physiological oxidation. In the animal body the oxidation of organic nitrogenous compounds (proteids, etc.) results in the production of urea and of other

similar substances no longer usable and presently cast off from the body. In plants the elimination of these products is more economically accomplished, for they furnish the foundations for the re-synthesis of albuminous compounds. These waste substances are removed by transforming them synthetically into useful compounds.

The non-nitrogenous substances which become oxidized are the fats and oils, the starches and sugars. The oxidation may first convert the hydrocarbons into carbohydrates, with the liberation of energy and the formation of by-products, carbohydrates and by-products then becoming still further oxidized with the liberation of still more energy. While respiration is going on, the other functions also in operation may involve the use, with chemical change, of some of each substance produced in respiration, and the formation in the cell of other substances not the products of respiration at all. It is therefore evident that to ascertain the material products of respiration is hardly less difficult than to determine the amount of energy liberated. Since each process is normal only when accompanied by all the processes going on at the same time, it is impossible to isolate any physiological process for purposes of study. The products of one set of chemical activities in the living body may enter wholly or partially, simultaneously or successively, into other chemical activities. The end products can be recognized and measured with comparative ease, but to tell exactly where or how they are formed is much more difficult and not now entirely possible.

Water and carbon-dioxide gas are the chief products of the physiological, as also of all other forms of combustion of carbon-containing bodies. They are formed whenever a sufficient amount of oxygen is united with the higher carbon compounds. In organisms living under such conditions that the air can penetrate to all their parts, enough oxygen will always be present for such complete decomposition. The oxygen does not unite of itself with the combustible compound, for even if active (nascent) oxygen is present at all, which seems improbable,[1]

[1] Pfeffer, W. *Pflanzen-Physiologie*, 2te Auflage, Bd. i (1897), p. 554. *Physiology of Plants*, translated by Ewart, vol. i (1900), pp. 545, 546.

it is present in amounts insufficient to accomplish the whole result. The union is accomplished by and in the living cell; whether with a more readily oxidizable substance first formed from sugar, or with sugar itself, is not now known. All that is known is that sugar, or some similar substance, and oxygen unite, forming as end-products mainly carbon-dioxide and water. The following reaction, without indicating what, if any, intermediate stages there may be, shows the material results:

$$n\,((C_6H_{12}O_6) + 6\,O_2 + \text{Aq.}) = n\,(6\,CO_2 + 6\,H_2O + \text{Aq.}).$$

(*Aq.*, representing the water in which the sugar is dissolved in the cell, does not enter the reaction. *n* indicates the unknown multiple of the minimum proportional formula $C_6H_{12}O_6$, which stands for the sugar molecule. The $6\,H_2O$ produced in the course of combustion may unite with the solvent water (*Aq.*), or may pass off as vapor, diffusing the faster from the cell by reason of the heat liberated).

Since other substances than sugar are also oxidized physiologically in the cell, other products will be formed, the kinds and the quantities of the latter varying according to the former. The commoner of these minor products are oxalic, malic, and citric acids, which accumulate in considerable quantities in certain plants (*e.g.*, in the leaves of *Oxalis acetocella*, in the *Crassulaceæ*, in apples, etc., and in the citrous fruits, lemons, limes, oranges, etc.), or are converted into salts (*e.g.*, calcic oxalate, crystallizing out of the solutions in which it is formed in the cell), or undergo other changes (*e.g.*, further oxidation).

In all organisms the oxidation of nitrogenous compounds, as well as non-nitrogenous, occurs in normal respiration. The proportional amounts of the two groups of compounds physiologically oxidized vary with different organisms. In the majority only organic and highly complex compounds are made to yield the needed energy, but in some much simpler inorganic compounds suffice, and in a few organisms already known the carbon compounds are not used at all.

The nitro-bacteria, as first shown by Winogradsky,[1] oxidize

[1] Winogradsky, S. Recherches sur les organismes de la nitrification, *Annales de l'Inst. Pasteur*, tomes iv, v (1889–91), and other papers.

simpler nitrogen compounds in order to liberate energy, employing carbon compounds only in the synthesis of food to be used in the construction of their own bodies. One set of nitro-bacteria oxidize ammonia, or compounds of ammonia, to nitrous acid, the first and last steps of the process being thus indicated:

$$2\,NH_4OH + 3\,O_2 = 2\,HNO_2 + 4\,H_2O.$$

Another set oxidize the nitrous acid, or its salts, to nitric acid, thus:

$$2\,HNO_2 + O_2 = 2\,HNO_3.$$

The sulphur bacteria (*Beggiatoa*, *Chromatium*, etc.) obtain most if not all of their kinetic energy by oxidizing sulphur compounds. They precipitate sulphur in their own bodies by oxidizing the sulphureted hydrogen present in the water in which they live.[1] If the supply of gas remain sufficient, the sulphur will accumulate as a reserve supply in the cells; if it decrease, the reserve sulphur will be oxidized and, uniting with water, will form sulphuric acid, or its salts, thus:

$$\begin{gathered} 2\,H_2S + O_2 = 2\,H_2O + S \\ S + O_2 = SO_2 \\ 2\,SO_2 + O_2 = 2\,SO_3 \\ SO_3 + H_2O = H_2SO_4 \end{gathered}$$

Those bacteria (*e.g.*, Crenothrix) which, living in water rich in iron, deposit iron in some form in or upon their own bodies, may obtain their kinetic energy by physiologically oxidizing ferrous compounds, presumably ferrous oxide, to ferric oxide.[2]

Other bacteria may be discovered which, needing carbon and nitrogen compounds only to supply the constructive elements of protoplasm, obtain their needed energy by oxidizing other substances present in solution in the waters in which they live.

[1] Winogradsky, S. Ueber Schwefelbakterien, *Botan. Zeitung*, 1887. *Beiträge zur Morph. u. Physiol. der Bakterien.* Leipzig, 1888. Miyoshi, M. Studien über die Schwefelrasen-Bildung und die Schwefelbakterien der Thermen von Gumoto bei Nikko, *Journ. Coll. Sci. Imp. Univ. Tokyo*, Bd. x, pt. ii, 1897.

[2] Winogradsky, S. Ueber Eisenbakterien, *Botan. Zeitung*, 1888. Molisch, H. Die Pflanze in ihren Beziehungen zum Eisen. Jena, 1892. Miyoshi, M. Ueber das massenhafte Vorkommen von Eisenbakterien in den Thermen von Ikao, *Journ. Coll. Sci. Imp. Univ. Tokyo*, pt. ii, 1897.

The essential product of respiration, the one which distinguishes respiration from all the other functions of the living organism, is kinetic energy. The material products vary in kind and in quantity according to the nature of the organism and the substances which can be affected, these substances being in most cases complex compounds elaborated within the body of the respiring plant, but not in all cases, as shown by the bacteria just mentioned.

Nor is free oxygen necessary to all organisms or to all cells. As the hæmoglobin of the blood is a complex compound from which some of the oxygen, only loosely held, can be readily withdrawn where oxidation for the supply of energy is needed, so the color products of certain bacteria (*e.g.*, *Bacillus bruneus*) are reserves of oxygen which become used when there is no longer an adequate supply of free oxygen.[1] From colorless compounds also, the cells at depths in the tissues of animals (perhaps also of plants[2]), to which free oxygen penetrates only in insufficient amounts if at all, obtain by decomposition the energy needed. These decompositions are not necessarily effected to secure oxygen for the oxidation of other substances, for the decompositions themselves release as kinetic the potential energy which was holding the complex substances together.

The mutual attraction of one atom of carbon and two of oxygen is so great as to make the molecule of carbon-dioxide very stable as well as very simple, for the affinities of the carbon and oxygen are satisfied. In the complex compounds of carbon, hydrogen, and oxygen in the sugar group, the affinities of the component elements are not satisfied ; the compounds are much less stable, as their ability to take up more oxygen shows. At ordinary temperatures and under ordinary conditions these compounds are stable. Their stability is due to the mutual affinities of their component atoms which exert an attraction upon one another sufficiently powerful to

[1] Ewart, A. J. On the Evolution of Oxygen from Colored Bacteria, *Journ. Linnean Soc.*, vol. xxxiii (1897), p. 123.

[2] Pfeffer, W. *Berichte d. math. phys. Klasse d. K. Sächs. Gesells. d. Wiss. zu Leipzig*, 27 Juli, 1896, p. 383.

hold them together in definite form. When the atoms are rearranged more compactly in simpler forms in space, their bonds or affinities are more completely satisfied, they unite more perfectly, oxidation takes place in the rearrangement, and energy is accordingly liberated and made available for other purposes. Energy is stored in the starch or oil or sugar molecule; the kinetic energy (solar or other) employed in the construction of the molecule remains in it as potential energy, holding the atoms together. The destruction of the molecule results in the liberation of so much kinetic energy as was employed in constructing it from the simple compounds worked upon.

The complete oxidation or combustion of a gram of dextrose (sugar), resulting in the formation of carbon-dioxide and water, as represented by this reaction,

$$C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + 6\,H_2O,$$

liberates 3939 small or ordinary calories,[1] or mechanical units of energy in the form of heat.[2] For the sake of obtaining these figures in more exact terms, for use in future comparisons, we may multiply this with the molecular weight of dextrose, thus:

$$\begin{array}{rlrl} \text{atomic weight of C} & = 12 & \text{of } C_6 & = 72 \\ \text{of H} & = 1 & \text{of } H_{12} & = 12 \\ \text{of O} & = 16 & \text{of } O_6 & = \underline{96} \\ & & C_6H_{12}O_6 & = 180 = \text{molecular weight of dextrose.} \end{array}$$

$$\begin{aligned} 3939 \text{ calories} \times 180 &= 709020 \text{ calories} \\ &= 709.02 \text{ Calories} \end{aligned}$$

The heat of combustion or complete oxidation of 1 gram-molecule, *i.e.*, of 180 grams, of dextrose, is then 709.02 great calories (C.).

This reaction, and the production of this amount of heat, take place only in the presence of sufficient quantities of free oxygen. Molecules more complex than those of carbon-dioxide

[1] A calorie (c.) is the heat required to raise 1 gram of water 1° C. in temperature; a great calorie (C.) is the heat required to raise 1000 gr. (1 Kilo) of water 1° C.

[2] Rechenberg, C. von. Ueber die Verbrennungswärme organischer Verbindungen, *Inaug. Diss.* Leipzig, 1880. See also Pembry, M. L., on Animal Heat, Schäfer's *Text-Book of Physiology*, vol. i (1898). Here the literature is fully given.

and water, though simpler than sugar, may be formed from sugar without free oxygen or with free oxygen in smaller proportions than 6 : 1. Complete oxidation (normal respiration) yields the largest amount of energy possible; less profound changes yield less energy. Thus the decomposition of sugar by yeasts, according to the following reaction, which represents only in simplest terms the nature of the chemical changes,

$$C_6H_{12}O_6 = 2\,C_2H_6O + 2\,CO_2,$$

forming without oxygen two molecules of alcohol and two of carbon-dioxide from one molecule of dextrose, yields only 67 calories per gram-molecule.[1]

The decomposition of one molecule of dextrose into one molecule of butyric acid, two of carbon-dioxide, and two of hydrogen, which is accomplished by a considerable number of species of bacteria, and may be represented by this reaction,

$$C_6H_{12}O_6 = C_4H_8O_2 + 2\,CO_2 + 2\,H_2,$$

yields about 75 calories per gram-molecule.[2]

Bacteria forming acetic acid, acting on dilute solutions of ethyl alcohol in the presence of free oxygen, partially oxidize the alcohol and decompose it into acetic acid and water, thus:

$$C_2H_6O + O_2 = C_2H_4O_2 + H_2O,$$

liberating 125 calories;[3] but if the alcohol were completely oxidized, as in ordinary combustion, the reaction would be

$$C_2H_6O + 3\,O_2 = 2\,CO_2 + 3\,H_2O,$$

and the heat liberated would be nearly three times as much, about 325 calories per gram-molecule.

In these figures we have indices of the relative values of complete and incomplete oxidations, and of oxidations and decompositions, as sources of energy in the form of heat. These figures are indices, to be trusted only so far as relative, not exactly proportional, values are concerned. The chemist can control all the conditions under which he makes a

[1] Rechenberg, *loc. cit.*, p. 66. [2] Rechenberg, *loc. cit.*, p. 67.

[3] Quoted from Berthelot in Biedermann's Chemiker-Kalender for 1897, p. 193 of the Beilage.

combustion in his laboratory and determines the number of heat units liberated; he can so regulate the process that there shall be no by-products, and that no other compounds are included in the reaction than those upon which he has determined to experiment. In the plant, on the contrary, other substances than dextrose may become oxidized, or the oxidation may be incomplete. In the laboratory one can deal with measured quantities of isolated substances; in the living organism indefinitely known quantities of many substance ogether are acted upon. Animal physiologists have done much more in this direction than have plant physiologists. The higher animals are better suited to such studies than are plants. The relatively high body-temperatures of warm-blooded animals permit direct temperature determinations from weighed quantities of known foods eaten, as well as calculations from the amounts of oxygen needed to effect combustions or decompositions. The animal physiologist can check the results obtained by one method with those reached through other methods. The results obtained by animal physiologists indicate that only about 95% of the calculated yield of energy from oxidation[1] appears as heat. So we must regard these figures as somewhat too high, but their suggestive value is great, whatever must be admitted as to their exact numerical value.

The larger organisms demand for the normal execution of their functions more energy than can be supplied by the rearrangement of the component atoms of the molecules always at hand. They must oxidize these molecules, and the more complete the oxidation, the greater the amount of energy liberated. Some of the smaller organisms supply themselves with adequate amounts of energy by the destruction of complex compounds within their own living cells. Probably some of the cells of all large multicellular organisms have recourse, at times at least, to the same means of securing needed energy, and when free oxygen is not obtainable, the majority of organisms can continue living for a time by so doing. From this the general inference may safely be drawn that the ability to obtain needed energy by the destruction of complex substances in the cells

[1] See Pembry in Schäfer's *Physiology*, vol. i, pp. 836, 837.

is inherent in all organisms, that in the majority of organisms and of their component cells this power is little needed and hence is practically undeveloped; but that, owing to the position of some cells deep in the tissues of many organisms and to the peculiar habits of some of the lowest organisms, these are obliged to obtain energy in this way and have developed their inherent power to a high degree.

Intramolecular respiration is the name given to this mode of respiration, a term not explicitly descriptive and therefore not entirely satisfactory. The German term *Spaltungsathmung* is in this regard more satisfactory, but it is not concisely translatable. Ordinary respiration is physiological oxidation or physiological combustion or aërobic respiration. It is dependent upon free oxygen and yields needed kinetic energy only by the union of free oxygen with combustible substances. Intramolecular respiration is physiological simplification of complex compounds or physiological rearrangement of atoms or anaërobic respiration. It takes place only when free oxygen is present in small quantity or is altogether absent. The results of the two processes are the same in kind — the liberation of the kinetic energy needed to continue living — but, as the figures quoted above show, not the same in degree.

Intramolecular respiration was first observed somewhat more than a hundred years ago by Rollo,[1] but only within the last few years has the connection between intramolecular and ordinary respiration been clearly demonstrated. Pasteur and other bacteriologists have contributed quite as much as animal and plant physiologists to our present knowledge of respiration. Pasteur and his followers have shown the peculiar habit of a large number of microörganisms of being active only when free oxygen is absent. When free oxygen is present, they are inactive, though they may remain alive. There is a chain of allied process: *first*, physiological respiration, or what may be called intramolecular respiration, the normal respiration of most organisms; *second*, physiological rearrangement of atoms into simpler molecules, intramolecular respiration, the mode of respiration to which many cells and even organisms

[1] Rollo. *Annales de Chimie*, t. 25, 1798.

have recourse under stress of circumstances; *third*, physiological rearrangement of atoms into simpler molecules, also intramolecular respiration, but the anaërobic normal respiration of a comparatively small number of invariably low organisms.

From experiments hitherto conducted, it would seem that the germinating seeds are better able to survive without a copious supply of oxygen than are the other parts of higher plants. This is what, *a priori*, might be expected, for the embryo in the seed, when it becomes active in germination, is a very vigorous organism, usually well supplied with just such foods as may be readily broken down into simpler compounds. The seeds of pea, for example, stimulated to germinate by being soaked for twelve to fifteen hours in water at room temperature, will continue to respire actively for forty-eight hours or more, even in a vacuum, producing carbon-dioxide in nearly the same quantity as under the same conditions of temperature, etc., in ordinary air. Of course some air containing free oxygen will be carried into the vacuum by the peas, but this will very soon be entirely exhausted in normal respiration. The continued supply of energy must be obtained by intramolecular respiration. Comparative investigations show that different plants and different organs vary considerably in their ability to substitute under stress intramolecular for normal respiration, and that in very few of the higher plants is intramolecular respiration, as measured by the yield in carbon-dioxide, so effective as normal respiration.

For all higher plants prolonged intramolecular respiration is impossible. To what this is due is not wholly clear. The substances first attacked in intramolecular respiration are the same as in normal respiration, *i.e.*, the sugars, starches (after conversion into sugar), and the fats and oils. Later the proteid substances enclosed in the cell, and finally the living substance itself, are decomposed to supply needed energy. Whether the cessation of intramolecular respiration in experiments upon higher plants, and the consequent death of the organism, are due to the destruction of part of the living substance, or to the production in the cells of poisonous substances, cannot now be determined. Certain it is that for

higher organisms intramolecular respiration is a function very limited in importance, taking place only when there is continued need of energy in the absence of free oxygen, and capable of being maintained for comparatively brief periods only. Like normal respiration, it is carried on solely by the living protoplasm, more or less actively according to the greater or lesser activity of the protoplasm. The substances decomposed are like those oxidized in normal respiration and differ in different species of plants. The products differ according to the plant, the conditions under which it acts, and the substances acted upon.

Alcohol may be produced in considerable amount. This suggests that in both fermentation and intramolecular respiration (if one may separate the two processes, for the former certainly includes the latter as well as nutrition) much of the chemical work may be done by enzymes produced by the respiring organism. Organic compounds and small quantities of many others may also be formed. In germinating peas, the alcohol produced may equal as much as 5% the weight of the moist seeds, enough to give some support to the idea expressed above, that the accumulation of the poisonous products of intramolecular respiration, as in fermentation, may cause the cessation of respiration and the death of the organism.

Between those plants for which aërobic respiration is indispensable to normally active life, and for which anaërobic respiration is only a means of maintaining life over unfavorable periods, and those for which anaërobic respiration is similarly and equally indispensable, there are all connecting stages. These are found among the lower plants, especially the fungi; but, as before stated, in all large multicellular organisms, especially among animals, there are probably cells, lying deep in the tissues, which are forced, by the positions they occupy, to supply themselves with needed kinetic energy by the same means as the anaërobic organisms, *i.e.*, by decomposing the complex compounds which they contain. There are then cells, as well as organisms, which are obligate aërobic, facultative aërobic, or obligate anaërobic. Obligate anaërobic cells and organisms live where the access of free oxygen is impossible or difficult and inadequate; for example, deep in living tissues,

either as component parts of these tissues or as parasites or saprophytes therein ; in the deeper layers of compact soils, in the mud of swamps and marshes, and in the ooze below bodies of comparatively still water, fresh and salt.

As in aërobic, so also in anaërobic respiration, other processes take place simultaneously with it. These, if not directly caused by respiration, are at all events maintained by the energy liberated in respiration and are so closely connected with it that to distinguish between the chemical products of respiration and those of the processes accompanying it, is a matter extremely difficult and still only imperfectly accomplished. Fermentation, decay, and disease at least accompany, if they are not actually a part of, the respiratory processes of certain low plants. Respiration, anaërobic as well as aërobic, is a function of the living protoplasm, which acts upon substances enclosed within its own body, producing simpler substances of which some remain in the respiring cell while others diffuse out of it. Some of the latter are chemically inactive, like carbon-dioxide and alcohol; others may act on substances outside the cell. In higher animals and plants the enzymes (*e.g.*, pepsin, diastase, etc.) are produced in connection with the process of nutrition, converting the substances upon which they act into available food compounds; but it is also certain that, among the enzymes produced, there are some which bring about such changes in the surrounding substances that these become available as sources of kinetic energy.

The diastase formed in the germinating seed, dissolving the starch deposited in the seed as a reserve food and converting it into sugar, makes the reserve food available for at least three purposes: *first*, for the construction of nitrogenous compounds (amides and proteids); *second*, for the formation of cell-wall (cellulose); *third*, for the liberation of energy by respiration. The production and action of this enzyme furnishes material for respiration, nutrition, and growth. The enzymes formed by lower plants are also useful in more than one way, not the least important use being the conversion of irrespirable into respirable substances.

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